PHOSPHOLIPID SURFACE BILAYERS AT THE AIR-WATER INTERFACE

I. Thermodynamic Properties

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ABSTRACT Dispersions of dimyristoylphosphatidylcholine (DMPC) in water spontaneously form a surface bilayer at the equilibrium air/water surface (Gershfeld, N. L., and K. Tajima, 1979, *Nature [Lond.]*. 279: 708–709). This phenomenon has now been demonstrated with dispersions of dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), and with a mixture of DMPC and DOPC. Each of these dispersions forms a surface bilayer at a singularity in temperature that is a characteristic of the phospholipid. The surface bilayer formed by the lipid mixture is shown to have the same composition as the bulk liquid-crystal phase of the dispersion, and the surface components have identical partial molar entropies as the bulk lipid components. These properties indicate that the surface bilayer has the same structure as the bilayer in the liquid-crystal phase of the bulk dispersion.

INTRODUCTION

The observation that dimyristoylphosphatidyl choline (DMPC)[1] dispersions in water spontaneously form a surface bilayer, i.e., a single bimolecular film at the air-water surface (Gershfeld and Tajima, 1979) is based on two independent sets of experimental data: a maximum in the surface pressure π_e-temperature phase diagram, and a corresponding maximum in the surface concentration of DMPC; both maxima occur at the same temperature, 29°C. The surface concentration at 29°C is equivalent to that of two condensed monolayers, and hence is called the surface bilayer. These results were first reported primarily to call attention to the phenomenon and to emphasize that lipid films may exceed monomolecular film densities. Subsequently, it was shown that the formation of the surface bilayer corresponds also to conditions for a higher order phase transition; the surface heat capacity-temperature relation appears as a lambda transition (Gershfeld, 1984).

Aside from the formal description of the surface bilayer, little has been said about the nature of this unusual state. Here we present general characteristics of the surface bilayer for a number of representative phosphatidyl cholines and a mixture of two of them. Thermodynamic arguments will be given to support our contention that the surface bilayer has a conventional bilayer structure. A second, accompanying study (Ginsberg and Gershfeld, 1985) will examine the water permeability of the DMPC surface bilayer. These results will demonstrate that the surface bilayer does indeed act as a substantial barrier to water permeation but only in a very narrow (±0.1°C) temperature interval. Future studies, now in progress, will examine the properties of other phospholipids and more complex mixtures.

METHODS

Materials

The synthetic samples of DMPC, DOPC, and DPPC (Applied Science Laboratories, State College, PA) used in this study contained trace amounts of lysolecithin and one other minor component that appeared at the front of the lecithin spot in thin-layer chromatography to the extent of ~1 mol %. Unpurified, these compounds yielded variable π_e data at temperatures below T_c, the gel–liquid-crystal transition temperature (Tajima and Gershfeld, 1981). Purification by gradient elution chromatography (chloroform/methanol [9:1] and methanol/chloroform [9:1]) of the lipid from a column packed with Unisil (100–200 mesh) (Clarkson Chemical Co., Williamsport, PA) yielded pure phosphatidylcholine, free of both contaminants. The purity was reflected in the reproducibility of the surface pressure-temperature phase diagram.

Tritiated DMPC and DOPC were gifts of Dr. R. E. Pagano (Carnegie Institution of Washington, Baltimore, MD). The ^{3}H-DMPC sample contained small amounts of fatty acid unsaturation plus breakdown products of autoradiolysis. The unsaturated fatty acid impurities were

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[1] *Abbreviations used in this paper:* DMPC, dimyristoylphosphatidylcholine; DOPC, dioleoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; π_e, equilibrium spreading pressure, surface pressure of lipid saturated solution; Γ, surface concentration, in moles per square centimeter.

eliminated by hydrogenating the sample, using Adam's catalyst. Gradient elution chromatography was used as the final purification step for both ^{3}H-DMPC and ^{3}H-DOPC.

All solvents used in this study were purified by passing them through columns of activated silica gel. Water was obtained from a quartz distillation apparatus; equilibrium water at pH 5.8 was used throughout.

Lipid dispersions in water were prepared by vortexing at temperatures slightly higher than T_c of the particular phospholipid. Radioactive phospholipid of any desired specific activity was prepared by dilution with nonradioactive phospholipid in chloroform solutions, followed by evaporation of the solvent under nitrogen. Further drying in vacuo had no effect on the surface radioactivity and, therefore, to minimize autoradiolysis this final stage of drying was not employed. The aqueous dispersion was prepared as described above.

Formation of Surface Films

Two methods were used to prepare equilibrium surface films. Neither involved the use of organic solvents for spreading the lipid on the water surface; thus possible artifacts introduced by the use of solvents (Horn and Gershfeld, 1977; Gershfeld, 1982) were avoided. In the first method, anhydrous material in excess of the amount required to form a monolayer was placed directly on the water surface, lipid spread spontaneously from the bulk lipid phase to cover the available water surface. This method is the basis of the equilibrium spreading pressure π_e measurement (Gaines, 1966; Gershfeld, 1976). The second method used a dispersion of the lipid in water. The dispersions gave reproducible surface pressures that were independent of the time of vortexing and of the amount of lipid added in the range of 1.0–0.01 mg/ml. Lipid concentrations of 0.2 mg/ml were used routinely.

Surface Pressure Measurements

The Wilhelmy plate technique was used to measure surface pressures (Gaines, 1966). Either a glass microscope coverslip (4 cm × 2.5 cm) or a platinum plate (1 cm × 2 cm) was used for the plate; the plate was suspended from a strain gauge (model G 110 B; Statham Instr., Puerto Rico) and positioned so that the edge of the plate just touched the water surface. The output from the strain gauge was amplified and recorded as a continuous function of time. A precision of ±0.2 dyn/cm for at least two independent measurements was routinely observed. The surface tension of water was found to be consistently within experimental error in agreement with reported values (Weast and Selby,1967). Measurements made with a horizontal float film balance confirmed results obtained with the Wilhelmy plate.

The procedures used for measuring surface pressures depended on whether films were formed by placing lipid on the water surface or by using dispersions of the lipid. In the former case values of surface pressure were obtained by first measuring the surface tension of water; then, with the plate still in contact with the water surface, lipid was placed there and the change in surface tension caused by the spreading lipid was recorded. When the surface pressure did not change by more than 0.1 dyn/cm for ~30 min it was assumed that equilibrium had been reached. Occasionally, some of the systems required overnight equilibration. It was concluded that equilibrium had been established if no further change in π_e was noted when additional solid lipid was added to the surface.

When lipid dispersions were used, adsorbed lipid film was removed from the surface of the suspension by aspiration with a clean Pasteur pipette attached to a vacuum line. Within 30 s after aspiration the Wilhelmy plate was positioned in the surface as described earlier, and adsorption of lipid to the air-water surface was followed with time. The earliest reading of surface tension usually was within 1 dyn/cm that of pure water, but it rapidly decreased as adsorption of lipid progressed.

The time to reach equilibrium surface pressure values with lipid suspensions was generally longer than when bulk lipid was placed directly on the water surface. For the dispersions, equilibration time increased as the concentration of lipid in the dispersion was decreased. A decrease in concentration from 0.2 to 0.012 mg/ml increased the time to reach equilibrium about fivefold (from 2 to 10 h at 30°C). Changing the temperature did not have significant influence on the time required to reach equilibrium. For the spreading pressure method the time to reach equlibrium depended on the amount of lipid deposited on the surface; equilibrium was usually reached within 2 h. Most of the surface pressure measurements were obtained by the more convenient spreading pressure technique and where both methods were used, agreement was within the experimental error. The main reason for using the dispersions was to demonstrate the equivalence of the two techniques, because dispersions were used to obtain the surface concentrations.

Surface films were formed in a cylindrical, water-jacketed Pyrex cell; the rim of the cell was lightly paraffined. The cell volume was 35 ml and the water surface area was ~18.4 cm^2. This cell was used to measure both π_e and Γ. Temperatures were monitored by means of a thermistor placed in the water surface of an identical cell in series with the cell used when either π_e and Γ measurements were made; constant temperature was maintained by circulating constant temperature water (±0.1°C) through the jackets of the cells. Measured surface temperatures depended on whether the cell was covered, on the position of the thermistor in the surface, the air temperature, evaporation, etc., and therefore there was some uncertainty of the actual temperature at the surface of the film. The measured surface temperature was usually ~0.5–1.0°C cooler than the bulk water temperature. However, we have used the measured surface temperature without any correction, since it was reproducible under identical experimental conditions and remained constant for the duration of each experiment.

Surface Concentration, Γ

Surface concentrations for lipid adsorbed from dispersions of lecithin was measured by using tritium-labeled compounds. Tritium radiation from the water surface was measured by a gas-flow thin window detector, using Q-gas (1.3% butane −98.7% He). The end window of the detector was composed of parylene (Spivak, 1970) with a vacuum deposit of gold; approximate thickness of the window was 70 μg/cm^2 parylene and 30 μg/cm^2 gold. The detector, constructed by the Aloka Co. (Mitaka, Japan) was used with a standard scalar (model 500; The Nucleus, Inc., Oak Ridge, TN). The detector covered the entire water surface of the glass cell. The counting efficiency of the detector was ~2% at a height of 1 mm above the water surface. This height was maintained for all experiments by using a Teflon gasket as a spacer between the detector and the rim of the temperature-controlled dish containing the lipid dispersion. The Teflon gasket was attached to the bottom of the end-window support ring of the detector and effectively eliminated evaporation of the aqueous dispersion.

Specific activities were obtained by depositing aliquots of the radioactive lipid in chloroform directly on the water surface of the constant temperature cell. This was the only time a solvent was used; all other films were formed by adsorption. Specific activity of the tritium-labeled phospholipids was ~35 Ci/mol, yielding counting rates of 1,000–10,000 cpm. Background was 100–300 cpm, starting with the lower value for a new thin window on the detector but increasing slowly with use. Background was measured just before and just after each measurement. Generally, background did not change significantly during a measurment. The radioactivity was measured with the detector in the identical geometry as when it was used for measuring the dispersion. Radioactivity was a linear function of the amount of lipid added to the surface, up to an upper limit that was approximately equal to the amount of lipid required for a condensed monolayer. This point was also recognizable by the persistence of the solvent lens on the water surface for as long as 1 to 2 min; at smaller volumes the solvent evaporated within a few seconds. The precision of the measurement of radioactivity was ±2.0%.

The procedure for the surface radioactivity measurement entailed filling the constant temperature water-jacketed glass cell to slightly above the lightly paraffined rim. After temperature equilibrium had been attained (~30–60 min), the dispersion was stirred, and the surface swept with a Teflon bar to remove adsorbed film and air bubbles that invariably

formed. The excess solution was removed by aspiration until the level of the liquid was brought to the rim of the cell. The cell was then carefully raised to the Teflon gasket of the detector and the counting rate measured until constant. The time to reach constant counting rate varied depending on the temperature and the amount of lipid in the dispersion; 30 min of constant counting rate was chosen to indicate that equilibrium was reached. The cell was then either emptied and additional lipid dispersion was added, or, more commonly, additional dispersion was added to raise the meniscus of the solution above the rim, and the solution was then stirred. The procedure described above was then repeated.

To obtain surface concentrations from the radiotracer measurements, it was necessary to separate the contribution of the bulk lipid dispersion to the total counting rate. For DMPC dispersions at temperatures below the gel–liquid-crystal transition (23.5°C), this correction was found to be unnecessary because of the low range of tritium radiation in water (~6 μm) and the high density of the lipid particles relative to water (Gershfeld, 1978). To illustrate this point, a dispersion of ^{3}H-DMPC was monitored at 16°C, below T_c; at this temperature the amount of DMPC adsorbed in the surface would be expected to be very low because the surface film is gaseous (Gershfeld and Tajima, 1977). Fig. 1 gives the total radioactivity as a function of time. The initial rise was due in part to an artifact that arises from the suction used to remove excess solution (see above). However, with time the surface radioactivity decreased to zero. The decrease in radioactivity in the early part of this experiment represented the settling of the dense lecithin particles out of the range of detection. As confirmation of this conclusion the final radioactivity was independent of the amount of lipid in the dispersion over a fivefold range of lipid composition. Thus at these temperatures the bulk contribution to the tritium radioactivity at the surface was zero.

At temperatures above T_c (e.g., 37°C), the surface radioactivity followed a different time course; instead of a decrease after the initial rise, the radioactivity increased slowly with time, reaching a maximum after ~20 min and remaining constant thereafter (Fig. 1). The slow increase in radioactivity followed approximately the time course of the increase in surface pressure for these systems, and therefore reflected the adsorption of lipid at the surface. The absence of a decrease in radioactivity with time, as noted with the dispersions at temperatures below T_c, suggests that the dispersion particles may not have settled out from the surface. Because we have not seen any significant dependence of surface radioactivity on the dispersion concentration in the range of 0.04–0.2 mg/ml, we have not applied by corrections for the bulk dispersion to the surface concentration. However, we estimate the maximum amount of the correction to be ~10% of the highest surface concentrations that we report.

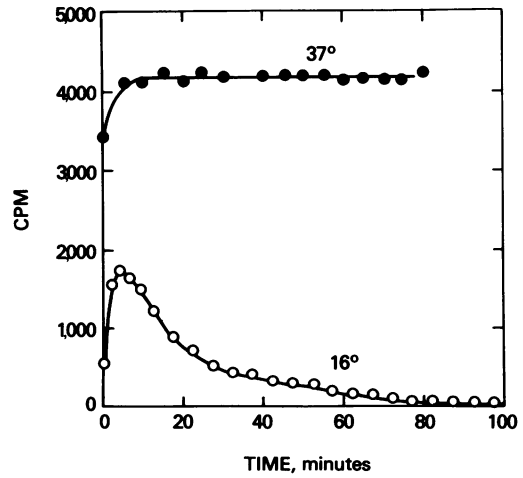

FIGURE 1 The surface radioactivity in counts per minute (CPM) is plotted as a function of time for dispersions of DMPC in water. T_c = 23.5°C.

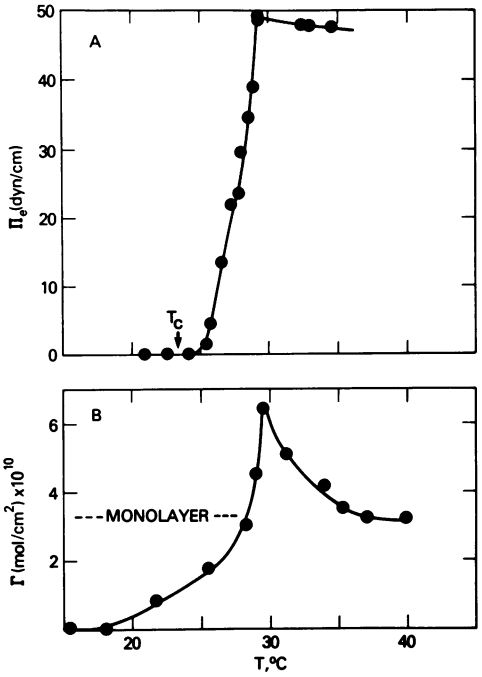

FIGURE 2 The surface properties of DMPC dispersions in water are shown as a function of temperature. (*A*) Equilibrium surface pressure, π_e. (*B*) Surface concentration, Γ. For condensed monolayers, A = 55 Å^2/molecule.

At temperatures that exceed 30°C, water condensed on the end window of the detector; this led to spurious counts. This problem was eliminated by warming the detector housing to the same temperature as the dispersion with a wrapping of electric heating tape.

RESULTS

For the initial report of the spontaneous formation of surface bilayers with DMPC dispersions in water, two independent measurements of the surface concentration were presented: a film-sweeping method and the radioisotope technique (Gershfeld and Tajima, 1979). Here the radiotracer method has been used exclusively. The data are presented in Fig. 2 where both surface pressure and surface concentration as functions of temperature are given for DMPC dispersions in water. Fig. 2 indicates parallel behavior for surface pressure and surface concentration: at temperatures below T_c (23.5°C), the bulk gel–liquid-crystal transition, both π_e and Γ are low; with increasing temperature both parameters increased monotonically to a maximum at 29°C. Above this temperature, maximum surface concentration decreased to a value at 37°C, which is equal to that for a condensed monomolecular film (55 Å^2/molecule), and remained relatively constant thereafter. Within experimental error the value of Γ at 29°C was twice that at 37°C. The surface concentration of DMPC at 29°C is therefore equivalent to two condensed monolayers, and hence has been called the surface bilayer. The surface concentration-temperature and surface pressure-temperature data of Fig. 2 were independent of the bulk concentra-

tion of lipid in the dispersion in the range of 0.04–0.2 mg/ml.

A significant feature of the data in Fig. 2 is the relatively steep slope of the surface pressure-temperature curve below the maximum at 29°C. For systems that undergo first-order phase transitions (either in the surface or in the bulk, dispersed state), values of $d\pi_e/dT$ yield differences in heats and entropies between the surface and bulk lipid states (Gershfeld, 1976, 1982). For the DMPC surface bilayer, the slope yields anomalously high values for these thermodynamic parameters (of the order of 10^5 cal/mol). We shall examine this anomaly in the Discussion.

Similar surface pressure-temperature behavior was observed with other phosphatidylcholines as seen in Fig. 3, where the results for dispersions of DPPC, DOPC, and for comparison, DMPC, are presented. For each of these systems the surface pressure reached a maximum at about the same value (49 dyn/cm), but at a temperature that was a characteristic of the lipid. The surface pressure maximum occurred at 7°C for DOPC, at 29°C for DMPC, and at 44°C for DPPC. In each case the temperature of the surface pressure maximum was above the gel–liquid-crystal transition temperature for the bulk dispersion. The values of $d\pi_e/dT$ at temperatures below the surface pressure maximum are all anomalously high.

These properties were also observed in a dispersion of an equimolar mixture of DMPC and DOPC. The temperature dependence of surface pressure and surface concentration for the mixture is presented in Fig. 4 *A*. The maximum surface pressure occurred at ~11°C, a temperature intermediate between the temperature of maximum surface pressure for the two pure components.

The corresponding surface concentration data for the mixture were obtained in two separate experiments: in the first, ^{3}H-DOPC + DMPC (1:1) was used in the dispersion

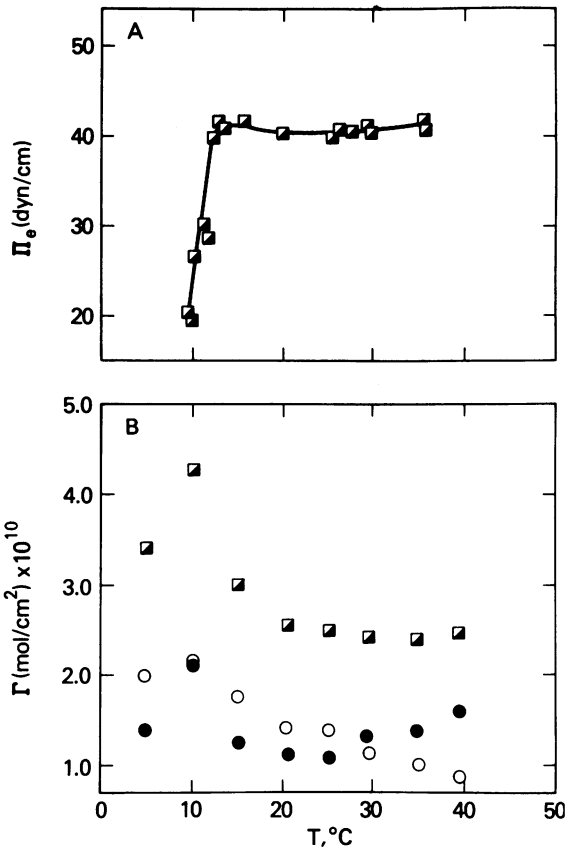

FIGURE 4 Surface properties of dispersion mixture of DOPC + DMPC (1:1) in water are shown as a function of temperature. (*A*) Equilibrium surface pressure, π_e; (*B*) surface concentration Γ. ○ indicates mixed dispersion using ^{3}H-DOPC + DMPC (1:1); ●, mixed dispersion using DOPC + ^{3}H-DMPC (1:1); and ◪, sum of ^{3}H-DOPC + ^{3}H-DMPC (1:1).

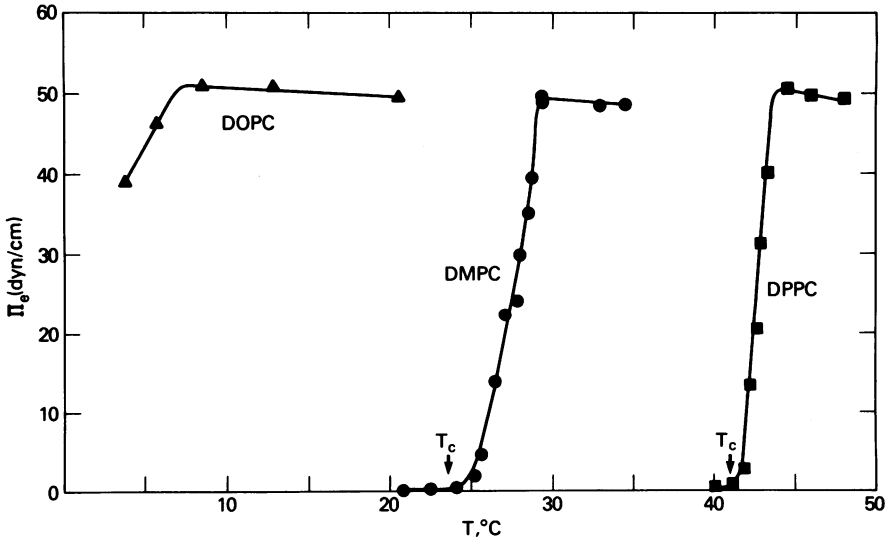

FIGURE 3 Equilibrium surface pressure π_e as a function of temperature for DOPC, DMPC, and DPPC dispersions in water. T_c for DOPC is −20°C.

so that only the DOPC contribution to the surface film was obtained. In the second, dispersions of DOPC + ^{3}H-DMPC (1:1) were used to obtain the DMPC contribution to the equilibrium surface film. The individual contributions were then added to obtain the total surface concentration in the surface film. The data, given in Fig. 4 *B*, indicate that the individual contributions to the surface film reached a maximum at ~10°C, and that the sum of the individual contributions was approximately twice the value of the condensed monolayer value of the mixture (e.g., at 30°C). Thus, even in mixtures of the phospholipids, surface bilayers form at a temperature that is characteristic of the mixture.

An important aspect of the mixed film is the fact that the composition of the surface bilayer was the same as in the bulk dispersion, whereas at temperatures below and above the surface bilayer temperature, the composition of the surface film differed significantly from that of the equilibrium dispersion. For example at 10°C, the surface bilayer consisted of an equimolar mixture of DOPC and DMPC, the same as that of the dispersion, but at 5°C and 15°C the equilibrium film mixture was at a ratio of DOPC/DMPC of ~3:2 instead of the 1:1 ratio in the bulk dispersion.

DISCUSSION

One of the major purposes of this study was to establish that the surface bilayer is an equilibrium structure and to characterize its properties in general terms, so that it may be readily identified in other systems. By establishing the surface bilayer as a general phenomenon we hope to dispel the natural inclination to reject the system outright because it seemingly contradicts the traditional concept that films are always monomolecular. Note that the concept of monomolecularity is only a useful model of film properties; no thermodynamic principles are violated if films exceed monomolecular densities.

In the discussion that follows we summarize the equilibrium criteria and general characteristics of surface bilayer formation; these are followed by a thermodynamic analysis of the system. Finally, arguments are presented to emphasize why we consider the structure of the surface bilayer to be similar to that of the lipid bilayer in the bulk dispersion.

Equilibrium Conditions for Surface Bilayer Formation

We employed the following experimental conditions as criteria for establishing that the surface bilayer is an equilibrium state: (*a*) the surface bilayer forms spontaneously by adsorption from a bulk dispersion of the lipid prepared by vortexing; (*b*) the surface concentration and surface pressure are independent of the bulk lipid concentration in the range 0.04–0.2 mg/ml; (*c*) equilibrium surface concentrations and surface pressures are independent of time; (*d*) when the equilibrium films are stirred and allowed to re-equilibrate, the same equilibrium values of surface concentration and pressure are obtained. On the basis of these criteria we assume that surface bilayers are equilibrium states.

Thermodynamic Properties of Surface Bilayers

The principal characteristics of the surface pressure- and surface concentration-temperature phase diagrams for DMPC dispersions (Fig. 2) are the maxima for π_e and Γ at 29°C, and the steep slope of the π_e-T curve at temperature below 29°C. Since DOPC and DPPC dispersions yield π_e-T phase diagrams similar to DMPC (Fig. 3), we conclude that all the lecithins form surface bilayers at the temperature of the surface pressure maximum.

Before analyzing the thermodynamic properties of surface bilayers, some of the features of the DMPC surface phase relations are examined to illustrate the unique characteristics of the surface bilayer state. The maximum in the surface pressure and in the surface concentration at 29°C (Fig. 2), properties of the surface bilayer, suggests the presence of a phase transition. However, it is not readily evident how the transition is to be represented and in the following discussion we consider several possibilities.

Generally, first-order surface phase transitions require that both the surface concentration and $d\pi_e/dT$ undergo a discontinuous jump at the temperature of the transition (Gershfeld, 1982). While $d\pi_e/dT$ appears to be discontinuous at 29°C, the surface pressure maximum, we note, however, that the surface concentration varies continuously with temperature throughout the entire interval of 26°–35°C (Fig. 2). We conclude, therefore, that surface bilayer formation is not a first-order transition.[2] Since $d\pi_e/dT$ can be discontinuous only at a first-order phase transition (Gershfeld, 1982, 1984), we may assume that this derivative is a continuous function of temperature over the entire temperature interval where the surface bilayer phenomenon is observed (26–35°C).

While the properties of the DMPC surface bilayer do not conform to the criteria for first-order phase transitions, higher-order phase transitions for the surface bilayer state are consistent with both surface concentration and $d\pi_e/dT$ as continuous functions of temperature. Thus, the surface heat capacity-temperature relation for DMPC dispersions resembles a lambda transition in the interval 26–35°C (Gershfeld, 1984). This suggests that surface bilayer formation may be represented by two higher order phase transitions, where at temperatures below 29°C the surface film is transformed from a condensed monolayer to the

[2]Calculation of the latent heat for a presumed first-order transition at 29°C (Eriksson, 1971; Gershfeld, 1976, 1982) yields an anomalous value of ~5×10^5 cal/mol, several orders of magnitude higher than expected for typical lipid phase transitions.

surface bilayer, and above 29°C the surface bilayer is converted to condensed monolayer at 35°C. In keeping with other higher order phase transitions (Ubbelohde, 1965), at temperatures where the surface concentration varies between the limits of that for a monolayer and a bilayer, the film is likely to behave as a hybrid of the two states over the temperature interval of the transition. In the study that follows, additional support for this model is presented (Ginsberg and Gershfeld, 1985). However, we recognize that other processes may also represent surface bilayer formation, but we present this model to emphasize that surface bilayer formation is not a first-order transition and that both surface concentration and $d\pi_e/dT$ are continuous functions of temperature.

Just as with single lipid dispersions, the equimolar DOPC-DMPC mixed dispersion forms surface bilayers at a temperature where the surface pressure passes through a maximum (Fig. 4). Since $d\pi_e/dT$ is a continuous function of temperature, we shall now prove that as a consequence of the surface pressure maximum, where $d\pi_e/dT = 0$, (*a*) a state of uniform composition is attained where the composition of the surface and bulk lipid become identical, and (*b*) the partial molar entropies of the surface bilayer and bulk lipid components are identical.

The proof follows the general outline of the analysis for systems that show extreme values of surface tension (Defay et al., 1966). We examine the system of two lipids dispersed as a homogeneous liquid crystal in water. The lipids are assumed to be poorly soluble in water, and therefore we need consider only the equilibrium between the liquid crystal and the surface film. The system contains three components: (1) water, (2) DMPC, and (3) DOPC. Since the system contains the air/water surface, it is under constant (atmospheric) pressure. At equilibrium the chemical potential of each component μ_i is constant throughout the system.

The Gibbs relation for $\pi_e = \pi_e(T, \mu_1, \mu_2 \ldots \mu_n)$ may be written

$$d\pi_e = S^\sigma dT + \Gamma_1 d\mu_1 + \Gamma_2 d\mu_2 + \Gamma_3 d\mu_3, \quad (1)$$

where S^σ is the entropy per centimeter squared of the surface film. For the liquid crystal phase, the Gibbs-Duhem relation is

$$S^{lc} dT + c_1 d\mu_1 + c_2 d\mu_2 + c_3 d\mu_3 = 0, \quad (2)$$

where S^{lc} is the entropy per milliliter of the liquid crystal, and c_i is the concentration (moles per milliliter) of each of the components in the liquid crystal. Solving Eq. 2 for $d\mu_1$ and substituting in Eq. 1 yields the following equation

$$d\pi_e = (S^\sigma - S^{lc}\Gamma_1/c_1)dT + (\Gamma_2 - \Gamma_1 c_2/c_1)d\mu_2 + (\Gamma_3 - \Gamma_1 c_3/c_1)d\mu_3. \quad (3)$$

For $d\pi_e/dT = 0$, the coefficients of the variables T, μ_2, and μ_3 must all be equal to zero. Thus

$$S^\sigma/S^{lc} = \Gamma_1/c_1 = \Gamma_2/c_2 = \Gamma_3/c_3. \quad (4)$$

If the only components in the system are those specified, i.e., no contaminants, and ignoring the minor components adsorbed from air, then Eq. 4 may be written in terms of the mole fractions (x_i) of each component in their respective phases

$$x_1^\sigma/x_1^{lc} = x_2^\sigma/x_2^{lc} = x_3^\sigma/x_3^{lc}. \quad (5)$$

Thus the composition of the surface and bulk liquid-crystal states are identical; this is a state of uniform composition. The thermodynamic analysis confirms the result obtained with the equimolar DOPC-DMPC dispersion (Fig. 4); at the temperature of the surface pressure maximum, surface bilayers form with a composition equal to that of the bulk dispersion.

Another consequence of the surface pressure maximum is that for each component the partial molar entropy in the surface bilayer, $\bar{S}_i^\sigma$, and in the bulk phase liquid crystal, $\bar{S}_i^{lc}$, are equal. This result is obtained by introducing the surface concentrations, $\Gamma_i = c_i S^\sigma/S^{lc}$ (Eq. 4), into the relation of the surface entropy expressed in terms of the partial molar entropies. Thus

$$S^\sigma = \sum_i \bar{S}_i^\sigma \Gamma_i, \quad (6a)$$

and for the liquid crystal

$$S^{lc} = \sum_i \bar{S}_i^{lc} c_i. \quad (6b)$$

Combining Γ_i (Eq. 4) with Eqs. 6a and 6b yields

$$\bar{S}_1^{lc} = \bar{S}_1^\sigma; \bar{S}_2^{lc} = \bar{S}_2^\sigma; \bar{S}_3^{lc} = \bar{S}_3^\sigma. \quad (7)$$

These results are general and the analysis may be applied to the DMPC dispersion as well.

Summary of Evidence that Surface Bilayer Structure Is that of a Typical Bilayer

Two observations have been presented that indicate the surface bilayer structure is that of a typical bilayer.

(*a*) The surface concentration is equivalent to approximately two condensed monolayers. The area per molecule compares favorably with values obtained for multilamellar bilayers of the lipids. Thus, the areas per molecule for DMPC and the equimolar mixture of DMPC and DOPC surface bilayers are 55 and 75 Å^2, respectively (Figs. 3 and 4 where $A = 1/\Gamma$). From x-ray diffraction studies (Lis et al., 1982) these areas are 65 and 73 Å^2 (obtained by taking the average of the values for DMPC and DOPC), respectively. Note that the surface bilayer areas may be slightly underestimated because of potential overestimation of the

surface bilayer density with radiotracers (see Methods); this correction may be ~5 Å^2 for typical bulk dispersion concentrations of 0.2 mg/ml. (*b*) The partial molar surface entropies are equal to the partial molar entropies of the bulk dispersion.

At the temperature of surface bilayer formation, the bulk lipid state consists of a stacked array of planar lipid bilayers (Lis et al., 1982). Given the equality of partial molar entropies in the surface and bulk lipid states and a surface density that is equivalent to a bilayer, it is reasonable to assign to the surface bilayer the conventional bilayer structure of the bulk dispersion. Other structures that combine monolayer and multilayer structures to yield a discrete surface density equivalent to a single bilayer would probably require a fortuitous arrangement of experimental conditions such as the amount of lipid in the dispersion. Our experiments indicate that the surface bilayer density is independent of the dispersion concentration. However, we recognize that by proposing the conventional bilayer structure for the surface bilayer, that we must also resolve questions pertaining to the mechanisms that limit the structure to a single bilayer. Since our experiments were not explicitly addressed to this problem, we defer a rigorous analysis of it, and have restricted ourselves here to a description of the phenomenon.

In summary, we have presented a systematic study of the surface bilayer phenomenon. It appears as a singularity in temperature forming a state of uniform composition where the composition of the surface is identical with that of the equilibrium bulk lipid phase. The surface components have partial molar entropies that are identical with the bulk lipid components. The surface density is equivalent to a single lipid bilayer. In the study that follows (Ginsberg and Gershfeld, 1985) we present the results of additional experiments that demonstrate that surface bilayers reduce the permeability of the surface to water, but only at the temperature where pure surface bilayer exists.

Received for publication 2 November 1983 and in final form 28 August 1984.

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